MICHIGAN DEPARTMENT OF ENVIRONMENT, GREAT LAKES, AND ENERGY

INTEROFFICE COMMUNICATION

TO: File for Cyanoguanidine (CAS No. 461-58-5)

FROM: Michael Depa and Rick Welsh, Toxicologists, Air Quality Division

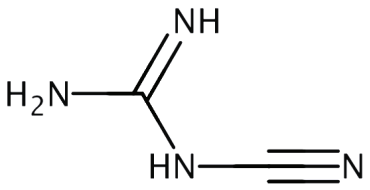
SUBJECT: Screening Level Derivation

DATE: September 20, 2023

In lieu of setting an Initial Threshold Screening Level (ITSL) for cyanoguanidine use the National Ambient Air Quality Standard (NAAQS) for Particulate Matter (PM) to evaluate emissions and impacts. See footnote No. 26 of the Toxics Screening Level Query Notes.

A literature review was conducted to determine an ITSL for cyanoguanidine. The following references and databases were searched to derive the screening level: European Chemical Agency (ECHA) Registration, Evaluation, Authorisation [sic] and Restriction of Chemicals (REACH), United States Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS), National Institute for Occupational Safety and Health (NIOSH®), American Conference of Governmental Industrial Hygienists (ACGIH®) Threshold Limit Values and Biological Exposure Indices (TLV/BEI) 2022 Guide, National Toxicology Program (NTP) Study Database, International Agency for Research on Cancer (IARC), Registry of Toxic Effects of Chemical Substances (RTECS®), Chemical Abstract Service (CAS) SciFinder®, PubMed®, EPA Computational Toxicology (CompTox) Database, EPA Provisional Peer-Reviewed Toxicity Values (PPRTVs), National Technical Information Service (NTIS), Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Level (MRL) database, and California Office of Environmental Health Hazard Assessment (OEHHA). NIOSH, ACGIH, EPA, ATSDR, and OEHHA have not derived health benchmarks for cyanoguanidine. Cyanoguanidine has a molecular formula of NH2(NH)CNHCN ($C_2H_4N_4$), and a molecular weight of 84.1 g/mol.

Figure 1. Molecular Structure of Cyanoguanidine



Physical Values

- Cyanoguanidine is a colorless solid that is soluble in water, acetone, and alcohol, but not nonpolar organic solvents (Güthner and Mertschenk, 2006).
- Cyanoguanidine has a water solubility of 32 g/L, which is classified as highly water soluble by the European Chemical Agency (ECHA, 2023).
- Vapor pressure is 2.7 mmHg at Temp: 25°C (CompTox, 2023).

The Food and Drug Administration (21CFR176.170) regulates cyanoguanidine such that it can be a component of paper and paperboard in contact with aqueous and fatty foods if it is used only:

1. As a modifier for amino resins.
2. As a fluidizing agent in starch and protein coatings for paper and paperboard.

Cyanoguanidine was investigated as an amendment to feed for grazing ruminants as a mitigation strategy to reduce nitrogen losses from grazed pastures (Welten et al., 2013).

The International Chemical Safety Card for cyanoguanidine states that, “The substance can be absorbed into the body by inhalation and by ingestion.” (ICSC, 2023)

Acute Inhalation Toxicity

A group of 5 male and 5 female Wistar rats were exposed for 4 hours to 259 mg/m³ cyanoguanidine in the form of dust (ECHA, 2023). Ninety-nine percent of the particle size ranged from 0.6-2.4 µm. During the first quarter hour of exposure the rats showed slight restlessness; during the remaining part of the exposure period, they were asleep. No deaths occurred during the 14-day observation period. The Lethal Concentration Zero or LC(0) is 259 mg/m³.

Subacute Duration Oral Toxicological Studies

Cyanoguanidine was not a skin sensitizer in a guineapig study at three concentrations after three weeks (Boman et al., 1985).

Groups of 4 male and 4 female beagles initially received cyanoguanidine (99.5% purity) in concentrations of 0, 10,000, 25,000 and 50,000 mg/kg diet corresponding to about 0, 330, 900 and 1750 mg cyanoguanidine/kg body weight and day for males; and 0, 350, 900 and 1600 mg cyanoguanidine/kg body weight and day for females for 4 weeks (MAK, 2007). Since the dogs did not eat enough food (due to vomiting), half of the animals were sacrificed after 4 weeks and the remaining 2 male and female animals were given cyanoguanidine in capsules in doses of 0, 250, 625 and 1250 mg/kg body weight per day for 22 days. None of the animals died during the study. Apart from emesis, no other clinical signs were detected. The body weight gain was lower by 33% and 64% compared with the control group in the male and female dogs of the highest dose group, respectively, between the 1st and 4th weeks. Thus, the feed consumption in this dose group was lower by 18% in the males and by 10% in the females. The analysis of clinical chemical and hematological parameters revealed sporadic, statistically significant changes in the form of increased hemoglobin and hematocrit values in the pretreatment period and reduced inorganic phosphorus, potassium, and

chloride concentrations after the 4-week treatment period, but the authors did not assess these effects as being due to treatment. The absolute and relative weights of the testes including the epididymides were slightly, but not statistically significantly reduced in 2 male dogs at 25,000 mg cyanoguanidine/kg diet and above. No gross-pathological or histopathological findings were reported. Significant shortcomings of the study included:

- The dosages varied considerably between the first part of the study and the bulk of the treatment period.
- The number of animals was limited in the later course of the study (2 dogs/group); and
- No gross or histopathological examinations were reported.

This study in beagle dogs cannot be used for the present assessment because of the significant deviations from standard toxicological study protocols.

In a 28-day range-finding study, cyanoguanidine was administered to groups of 10 male and 10 female Wistar rats in doses of 0, 200, 2000 and 20,000 mg/kg diet (MAK, 2007). The doses of cyanoguanidine were reported as 0, 23, 240 and 2350 mg/kg body weight per day for males and about 0, 24, 240 and 2400 mg/kg body weight for females. Doses were determined based on the body weight and feed consumption in the middle of the study. The rats revealed no clinical signs/observations. No substance-induced alterations were observed in the low and intermediate exposure groups. The increased body weight of the females of the intermediate exposure group (240 mg/kgBW/day), was interpreted by the authors as an incidental finding. The authors stated that the increased body weight was probably responsible for the statistically significant increase of the absolute, but not of the relative kidney weight. The body weight of the males was slightly, but not significantly reduced in the high exposure group (2350 mg/kg BW/day). Water consumption was significantly elevated in the males compared with the control group, whereas feed consumption revealed no substantial variations among the groups. The analysis of the hematological parameters showed a statistically significant decrease of the leukocyte count in the males in the 2,350 mg/kg BW/day group, which according to the authors, was due mainly to an extremely low value in one of the treated animals of this exposure group. When this value was ignored in the calculation, the leukocyte count was still reduced, but not statistically significantly compared with the control. No alterations were detected either by gross pathology or in the histopathological examination of the liver and kidneys. The NOAEL (no observed adverse effect level) specified by the authors is 2000 mg cyanoguanidine/kg diet corresponding to about 240 mg cyanoguanidine/kg body weight per day.

A short communication reported that the 37-day administration of 10,000 mg cyanoguanidine/kg diet to dogs caused no toxic effects (MAK, 2007). Compared with the control group, no abnormalities were detected for body weight or feed consumption, or in the hematological analysis or for the gross-pathological or histopathological examinations.

A field trial and laboratory incubation study were conducted to examine the effects of oral administration of cyanoguanidine to non-lactating Friesian dairy heifers on excretion of cyanoguanidine in urine and efficacy in soil (to reduce nitrogen losses from grazed pastures) (Welten et al., 2013). Dairy heifers were orally administered cyanoguanidine daily at three treatment levels (low, medium, and high; 12, 24 and 36 g cyanoguanidine per heifer per day) and compared to a cyanoguanidine control group over a 90-day continuous dosing period. There were no adverse effects of cyanoguanidine administration on heifer health or growth, as inferred by live-weight gain and measured blood metabolite levels.

Mature non-lactating, Friesian or Friesian-cross cows (2-7 years old) were fed cyanoguanidine at daily doses of 0 mg/kg (n=15), 150 mg/kgBW/day (n=31), 450 mg/kgBW/day (n=21) and 750 mg/kgBW/day (n=12) (Cayzer et al., 2017). Animal body weights determined prior to the start of treatment then every subsequent fortnight during the trial were used to calculate individual doses. Daily health observations were carried out on each cow until Day 86 of the study. On Day 28 one cow from the 750 mg/kgBW/day dose group was observed with signs of toxicity, and subsequently toxicity was noted in other cows. Clinical signs in the first case included depression, pyrexia (40.9°C), salivation and dehydration, in addition to progressive weight loss, followed by death on Day 32. Other cows from all treatment groups developed clinical signs of toxicity resulting in euthanasia of seven animals. Toxicity occurred in 7/31 (23%) cows in at 150 mg/kgBW/day, 11/21 (52%) cows at 450 mg/kgBW/day, and 10/12 (83%) cows at 750 mg/kgBW/day (see Table 1, next page). No clinical abnormalities were observed in any of the control cows. The authors defined “clinical signs of toxicity” as follows:

> Dermatitis (alopecia, skin thickening, pruritus); petechia of the mucous membranes; neutropenia and/or thrombocytopenia on more than one blood sampling occasion; pyrexia (rectal temperature >39.1°C) with weight loss and at least one occurrence of neutropenia and/or thrombocytopenia.

Gross pathological findings included generalized lymphadenopathy, subcutaneous edema, petechiation of mucosal and serosal surfaces, and gastrointestinal hemorrhage. Histologically, multiple organs and tissues contained inflammatory foci characterized by infiltrates of lymphocytes, plasma cells, macrophages and occasionally prominent multinucleated giant cells and eosinophils.

Table 1. Number of Cows Affected by Cyanoguanadine (Cayzer et al., 2017)

	Dose (mg/kgBW/day)		
	150	**450**	**750**
Number of Cows in Each Group	31	21	12
Died or subjected to euthanasia	4 (13%)	2 (10%)	2 (17%)
Showing clinical signs	7 (23%)	11 (52%)	10 (83%)
Specific clinical signs:			
Pyrexia	4	5	7
Pruritus and alopecia; predominantly head and neck	6	7	1
Petechial hemorrhages	3	3	6
Weight loss >14 kg/2 weeks	6	3	4
Oral or nasal discharge increased or mucopurulent	5	3	3
Increased lung sounds	4	5	2
Depression	4	3	2
Reduced gut sounds/abdomen appeared empty	4	2	3
Pale mucous membranes	3	1	4
Congested mucous membranes	1	4	1
Oral or nasal erosions	2	0	2
Dehydration	2	0	1
Diarrhea	1	1	0
Abortion	2	0	2

In response to the initial cases of sickened cows that required euthanasia, cyanoguanidine treatment was discontinued first in the 750 mg/kgBW/day group (on Day 34), followed by the 450 mg/kgBW/day group (Day 59) and finally in the 150 mg/kgBW/day group (Day 64). Signs of disease continued to manifest in treated cows up to 20 days following the last administration of cyanoguanidine. A LOAEL of 150 mg/kgBW/day is identified based on findings in Table 1.

Subchronic Duration Oral Toxicological Studies

Male and female F344 rats were fed diets containing 1.25, 2.5, 5, and 10% cyanoguanidine for 13 weeks (Matsushima et al., 1991). No animals died during the administration period. Inhibition of body weight gain was more marked in both sexes of the 10% group and in females of the 5% group as compared with the control group. Mean food intake in males of the 5% and 10% groups and in females of the 10% group was significantly higher than that in the control group. Serum biochemistry evaluations revealed a higher level of serum blood urea nitrogen in both sexes of the 10% group. The authors stated that toxic changes were characterized by the occurrence of intranuclear eosinophilic inclusion bodies in the proximal tubular epithelium of the kidney in both sexes of the 10% group. Similar inclusion bodies were also seen in 2 out of 10 males of the 5% group.

In a 13-week range-finding study, groups of 10 male and 10 female F344 rats received cyanoguanidine in the diet in concentrations of 0, 12,500, 25,000, 50,000 and 100,000 mg/kg diet corresponding to about 0, 570, 1250, 2600 and 5800 mg/kg body weight and day for males and 0, 700, 1500, 3000 and 6800 mg/kg body weight and day for females based on the body weight at the end of the study and the overall substance absorption throughout the study period. No animal died during treatment. Initial effects occurred in the male and female rats at 12,500 mg/kg diet and above. The platelet count was increased in both sexes, the leukocyte count was increased only in the males, and the urea concentration and the aspartate aminotransferase activity were elevated only in the female rats. In addition, the absolute liver weight was reduced in female rats. Increased feed consumption and a decrease in the serum concentrations of total protein, total cholesterol and calcium and in the alanine aminotransferase activity were recorded in male rats at 50,000 mg/kg diet. Females revealed reduced body weight, which led to a reduced absolute thymus weight, and their relative brain and heart weights were increased. A lowering of the total serum protein concentration and an increase in alkaline phosphatase activity were also detected. At 100,000 mg/kg diet, the organ weights of heart and kidneys were changed in the males in accordance with the reduced body weight. The absolute and relative brain weights and alkaline phosphatase and cholinesterase activities increased. Feed consumption was higher in the females, and the relative spleen and kidney weights were increased. The concentration of total cholesterol in the serum was reduced. Histopathologically, intranuclear inclusion bodies in the proximal tubular epithelium of the kidneys, which in 2 of 10 males, occurred even at 50000 mg/kg diet, were diagnosed in both sexes.

In a 13-week study, cyanoguanidine (100% pure) was administered to 10 male and 10 female Sprague-Dawley rats with the diet. The doses were 0, 240, 2400, 8000 and 24,000 mg cyanoguanidine/kg diet, corresponding to a mean cyanoguanidine uptake of about 0, 16, 150, 550 and 1600 mg/kg and day for males and of about 0, 18, 200, 650 and 1900 mg/kg body weight and day for females. A satellite group of 10 male and 10 female Sprague-Dawley rats, which were given 24,000 mg cyanoguanidine/kg diet, was examined 4 weeks after the end of treatment. The survival rate, clinical parameters, body weight, organ weights of liver, kidneys, adrenals and testes including epididymides and the opthalmological findings yielded no deviations between the control and treated animals. Feed consumption was only statistically significantly increased in the female rats treated with 2400 and 24,000 mg cyanoguanidine/kg diet. The authors did not regard this change or the increase in total protein and albumin concentrations in male rats (2400 mg cyanoguanidine/kg diet) as being induced by the test substance. The gross pathological examination showed no changes in the treated groups compared with the control group. Findings such as uterine dilation, mononuclear infiltrations in the mucosa of the urinary bladder, hydronephrosis and chronic progressive nephropathy were detected by histopathology, but these were not statistically significant and were not interpreted by the authors as being related to the test substance. The authors concluded that the NOAEL is above 24,000 mg/kg diet, corresponding to about 1600 mg/kg body weight and day for male rats and 1900 mg/kg body weight and day for female rats.

A short communication reported that the 26-week administration of 10,000 mg cyanoguanidine/kg diet to rats caused no toxic effects (MAK, 2007). Compared with the control group, no abnormalities were detected either for body weight, feed consumption and the results of hematological determinations or for the gross-pathological and histopathological examinations.

Chronic Duration and Carcinogenicity Oral Studies

The investigation of the chronic toxicity of cyanoguanidine (> 99.8% purity) in Sprague-Dawley rats was combined with a study for carcinogenicity (MAK, 2007; SKW, 1992a). In the chronic toxicity study, 20 rats per sex were treated with 50,000 mg cyanoguanidine/kg diet corresponding to 1980–5200 mg/kg body weight (BW) per day (mg/kgBW/day)(male rats) and 2850–6350 mg/kgBW/day (female rats) for 52 weeks. The corresponding control group consisted of 10 animals per sex. In the carcinogenicity study, groups of 50 male and 50 female rats received 0, 5000, 15000 and 50,000 mg cyanoguanidine/kg diet corresponding to about 0, 170–490, 540–1480 and 1740– 5110 mg/kgBW/day (males) and 0, 210–580, 690–1760 and 2420–6370 mg/kgBW/day (females) for 104 weeks. There were no differences between the groups treated with cyanoguanidine and the control group for the survival rate, feed consumption, the opthalmological findings or the results of the thyroid function tests. According to the authors, the clinical signs observed are not related to the treatment with cyanoguanidine. Effects were recorded only in the animals of the high exposure groups (50,000 mg cyanoguanidine/kg diet) both in the carcinogenicity and in the chronic toxicity study. The body weight gains were statistically significantly reduced. The organ weight changes were probably due to the altered body weight – except for a statistically significant decrease in the relative liver weight and an increase in the relative adrenal weight. No histopathological findings were obtained. Changes in the clinical chemical and hematological parameters were not consistent between the chronic toxicity study and the carcinogenicity study or were not dose-dependent or were not observed at all, but only at some times of examination. Therefore, the authors do not regard the effects observed as being induced by the substance. Only the blood urea value in the male rats of the chronic toxicity and carcinogenicity study (50,000 mg/kg diet) was increased in animals of both groups after 26 and 52 weeks. The authors concluded the NOAEL to be 15000 mg cyanoguanidine/kg diet corresponding to about 540–1480 mg/kgBW/day for male rats and 690–1760 mg/kgBW/day for female rats. It is not clear why the authors provided a range of doses per body weight and did not calculate an overall study-duration average dose per body weight per day for each group. To facilitate a quantitative risk assessment, it was deemed reasonable by the Michigan Department of Environment, Great Lakes, and Energy to assume that the midpoint of the range represents a central estimate of dose over the lifetime of the rats; therefore, the NOAELs of 940 mg/kgBW/day and 1070 mg/kgBW/day were assigned to male and female rats, respectively.

In a carcinogenicity study, groups of 50 male and 50 female F344 rats received 0, 25,000 and 50,000 mg cyanoguanidine/kg diet corresponding to about 0, 850 and 1870 mg/kgBW/day (males) and about 0, 1200 and 2350 mg/kgBW/day (females) for 104 weeks (Yasuhara,1997). The animals were observed up to the 113th week.

Histopathology result of tissue examinations did not reveal increase incidence of tumors. Survival rate yielded no differences between the control group and the treated groups. The dose-dependent decrease in body weight gain was statistically significant in the rats treated with 50,000 mg cyanoguanidine/kg body weight. Within a 4-week treatment-free period after substance administration had ended, the animals of both exposure groups showed a considerable body weight gain compared with the control group. Feed consumption did not differ in the control or the treated animals. In the highest exposure group (50,000 mg/kg diet), a statistically significant increase of slight bile duct hyperplasia was detected in the females and intranuclear eosinophilic bodies in the proximal tubular epithelium of the kidney were observed in the males. Because of the low number of parameters examined and insufficient documentation, the study can be assessed only with reservations. The LOAEL is 2350 mg/kg based on bile duct hyperplasia in females. The NOAEL is 1200 mg/kg. The lesions observed in the male F344 rat in the renal proximal tubular epithelium are not relevant to human risk assessment (EPA, 1991).

Developmental/Reproductive Oral Toxicity

Groups of 22 pregnant New Zeeland White Rabbits were dosed by gavage with 0, 200, 400 and 1000 cyanoguanidine mg/kg/day from Day 6 (G 6) to Day 28 (G 28) of gestation inclusive (22 days) (ECHA, 2023). One female in the 400 mg/kg/day group and one female in the 1000 mg/kg/day group had red traces in the cage on several occasions, which was possibly associated with resorption of the implantations because these females had no viable fetuses at necropsy. Overall mean body weight gain in the 1000 mg/kg/day group was statistically significantly higher (+41 %) than the control during the dosing period (G 6 to G 29). There was no effect of treatment on mean gravid uterus weight in any group. One, 1 and 2 of these females in the control, 400 and 1000 mg/kg/day groups, respectively had no viable fetuses. Post-implantation data: There was no treatment-related effect on embryo-fetal survival in any group. One or 2 female(s) in each of the control, 400 and 1000 mg/kg/day group had 4 to 7 early or late resorptions and no viable fetuses. One female in the 1000 mg/kg/day group had a dead fetus. Mean live litter size was comparable in all groups. Evidence of potential embryo-fetal effects of cyanoguanidine was restricted to slightly higher incidences of fetuses with an absent interparietal bone, unossified phalanges of the forepaw and 5th sternebrae, and variations in the number of full ribs (n=12 unilaterally or bilaterally) in the 1000 mg/kg/day group compared with the concurrent control and historical control data. A similar trend was noted for the interparietal bone only in the 400 mg/kg/day group. In total, there were 1 (1), 5 (4), 3 (3) and 2 (2) fetuses (litters) with malformations in the control, 200, 400 and 1000 mg/kg/day groups, respectively, none of which were attributed to cyanoguanidine. The principal changes included sternebral defects for 4 fetuses in the 200 mg/kg/day group and 2 in the 400 mg/kg/day group. However, the authors stated that there was no similar finding in the 1000 mg/kg/day group and these changes are similar to the historical background for the strain of rabbit; therefore, there was no association with exposure to cyanoguanidine. The authors stated that the other malformations noted (hyperflexion or malrotation of a forepaw, great blood vessel changes, vertebral defects leading to scoliosis, a heart defect and omphalocele) were considered to be incidental since they are part of the background of changes noted in

the strain of rabbit, the incidence did not increase in a dose-dependent manner, were isolated findings, and there was a lack of any associated increases in embryo-fetal death and in less severe structural changes. A LOAEL of 1000 mg/kgBW/day was identified based on higher incidences of fetuses with an absent interparietal bone, unossified phalanges of the forepaw and 5th sternebrae, and variations in the number of full ribs (n=12 unilaterally or bilaterally). A NOAEL of 400 mg/kgBW/day was identified in this reproductive/development study.

In a 1990 range finding prenatal developmental toxicity study cyanoguanidine (99.5%) was administered to 6 female Crl:CD BR rats/dose by gavage at dose levels of 0, 250, 500, 1000, or 2000 mg/kgBW/day from days 6 through 15 of gestation (ECHA, 2023). Oral gavage administration of cyanoguanidine in Crl:CD BR rats during gestation days 6-15 did not result in either maternal or embryo/fetal toxicity at doses up to 1000 mg/kg. No indications of an adverse effect on fetal development were seen at any dose tested. Maternal and embryo/fetal toxicity were indicated by slightly lower body weights in the highest dose group receiving 2000 mg/kgBW/day. No indications of an adverse effect on fetal development were seen at any of the doses tested. The maternal LOAEL is 2000 mg/kgBW/day, based on body weight changes. The author's maternal NOAEL is 1000 mg/kgBW/day. No indications of an adverse effect on fetal development were seen at any of the doses tested. The author's developmental NOAEL is 2000 mg/kgBW/day. ECHA Remarks: Major deficiencies of the study were the relatively small group size of 6 females and the lack of an investigation of skeletal or soft tissue alterations. ECHA (2023) remarked: Only 6 females per group; food consumption not recorded; body weight recorded only every 4 days not 3 days; only external alterations, no skeletal or soft tissue alterations investigated. This is described as a range-finding study by ECHA.

In a 1990 2-generation reproduction oral toxicity study in rats, cyanoguanidine (99.5%) was administered to Crl:CD BR rats/sex/dose in diet at dose levels of 0, 5000, 15,000, and 50,000 ppm. There were 26 animals per sex per dose: F0 and F1 animals (ECHA, 2023). Dietary exposure of rats to cyanoguanidine in a 2-generation study resulted in consistent and significant differences in body weights at the 50,000 ppm as well as a slight reduction in fertility and pregnancy rates. The NOAEL in this study is considered to be 15,000 ppm (ca. 725 mg/kgBW/day in male rats (week 13-14)). These effects can be attributed to overt toxicity. There was a minimal reduction of mean body weight at the 15,000 ppm level, but the overall weight change during the growth period was not statistically significant. There were no effects on body weight at the 5000 ppm level and no adverse effects on reproductive performance at the 5000 or 15,000 ppm diet levels. No effects on pup viability were noted at any dose level tested. Based on body weight changes the LOAEL is 50000 ppm diet, or 2615 mg/kgBW/day in males, and 3551 mg/kgBW/day in females. The author's NOAELs are 725 mg/kgBW/day in males, and 1002 mg/kgBW/day in females.

Discussion
Only one inhalation study was identified that evaluated cyanoguanidine toxicity. An acute inhalation study (4-hour exposure) showed that 259 mg/m³ cyanoguanide produced slight restlessness then sleep, but no deaths after a 14-day observation period.

Subchronic and chronic duration oral studies showed LOAELs and NOAELs of approximately 2000 and 1000 mg/kgBW/day, respectively.

Cyanoguanide fed to dairy cattle exhibited effects as low as 150 mg/kgBW/day. A NOAEL was not identified from the cattle study. Cattle have a pre-stomach in which bacteria break down cellulose. Because the presence of a rumen is not present in human and cyanoguanidine was administered in the feed to the dairy cattle, it is likely that cyanoguanidine toxicity in cattle is not directly comparable to potential toxicity in the human. Therefore, the dairy cattle study is not recommended for human health risk assessment of oral cyanoguanidine exposure.

A developmental LOAEL of 1000 mg/kgBW/day was identified with observations of higher incidences of fetuses with an absent interparietal bone, unossified phalanges of the forepaw and 5th sternebrae, and variations in the number of full ribs (n=12 unilaterally or bilaterally). A developmental RfD can be generated from the NOAEL of 400 mg/kgBW/day identified from this study. A route-to-route conversion from oral to inhalation is appropriate because cyanoguanidine is expected to be 100% absorbed via the inhalation tract (ECHA, 2023).

Screening Level Calculation
A Reference Dose (RfD) can be calculated from the developmental study in New Zeeland White Rabbits (ECHA, 2023) where a NOAEL of 400 mg/kgBW/day was identified. To calculate the human equivalent dose of the rabbit NOAEL the dosimetric adjustment factor (DAF) is calculated according to EPA (2011).

$$DAF = (BW_{rabbit})^{0.25}/(BW_{human})^{0.25}$$

Using default weights of 2.0 kg for 52 day old female New Zealand Rabbits (EPA, 1988) and 70 kg for humans yields a DAF of 0.41.

$$DAF = (2.0)^{0.25}/(70)^{0.25}$$
$$DAF = 1.189/2.893$$
$$DAF = 0.41$$

The human equivalent dose is calculated using the DAF as follows:

$$NOAEL_{human} = NOAEL_{rabbit} \times DAF$$
$$NOAEL_{human} = 400\ mg/kg \times 0.41$$
$$NOAEL_{human} = 164\ mg/kg$$

The $NOAEL_{human}$ of 164 mg/kg was used as the point of departure (POD) for derivation of the RfD. A total uncertainty factor (UF_{TOT}) of 30 was used as described below.

$$RfD = POD/(UF_1 \times UF_2)$$

Where

UF1 = 10 for intraspecies (sensitive individuals), and
UF2 = 3 for interspecies (animal to human); reduced from 10 to 3 when DAF is used to convert from animal to human dose (human equivalent dose or HED).

Then, the RfD is calculated as:

$$RfD = (164 \text{ mg/kg})/(10 \times 3)$$
$$RfD = 5.47 \text{ mg/kg}$$

Pursuant to Rule 232(1)(b) the ITSL is calculated as follows:

$$ITSL = RfD \times 70 \text{ kg}/20 \text{ m}^3 \times \text{unit conversion}$$
$$ITSL = 5.47 \text{ mg/kg} \times 3.5 \times 1000 \text{ µg/mg}$$
$$ITSL = 19{,}100 \text{ µg/m}^3$$

Rounding the candidate ITSL to one significant figure yields a candidate ITSL of 20,000 µg/m³. Because the Preliminary ITSL was derived from a short-term developmental study, a 24-hour averaging time is appropriate, pursuant to Rule 233(2). However, because cyanoguanidine is a solid it is appropriate to use the NAAQS for Particulate Matter to comply with Rule 225[1]. Footnote No. 26 (see below) describes additional information regarding cyanoguanidine.

> 26. This toxic air contaminant (TAC) is reasonably anticipated to exist as a particle in the ambient air. A toxicological review has determined that, in lieu of setting a screening level, the primary NAAQS for particulate matter (PM2.5, PM10) are reasonable and appropriate health protective levels for the particulate. The combined ambient impact of all particulate TAC emissions from the process must be below the applicable PM primary NAAQS (PM2.5, PM10). The PM primary NAAQS for particulate matter may be used in permit to install exemption determinations for this TAC under Rule 290(2)(a)(iii) or Rule 291.

The NAAQS for PM is to be used in lieu of an ITSL.

[1] Rule 336.1225 et. seq. of the Michigan Administrative Code promulgated pursuant to Part 55, Air Pollution Control, of the Natural Resources and Environmental Protection Act, 1994 PA 451, as amended (NREPA).

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